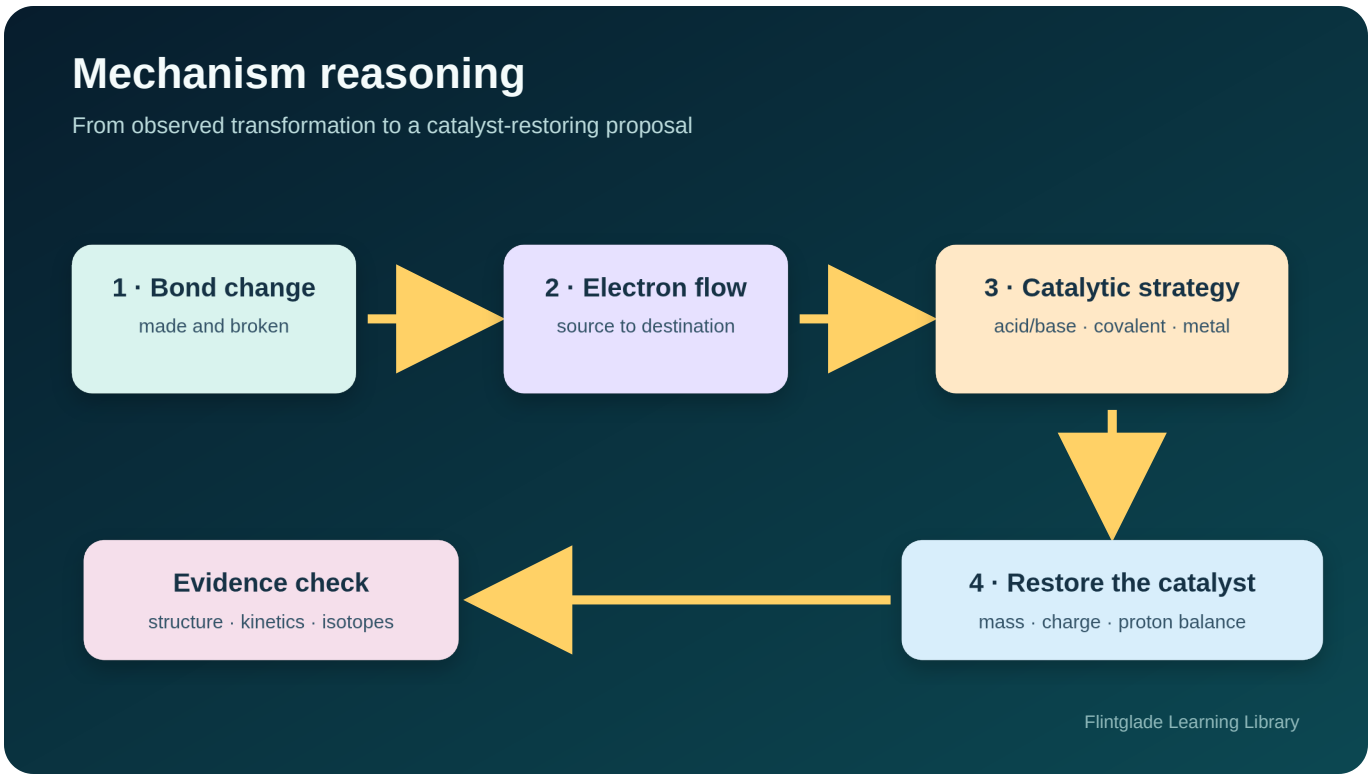
Organic Reaction Logic in Biochemistry

Flintglade Learning Library · General Biochemistry

Audience: Readers translating organic-chemistry ideas into enzyme and pathway mechanisms.

MCAT connections: This guide can assist study of amino acids and proteins, enzymes and kinetics while keeping general biochemistry as its primary frame.



About this edition: This standalone edition organizes the material for independent reading with clear navigation, reusable reasoning frameworks, and print-ready presentation.

Learning goals

- Recognize nucleophiles, electrophiles, and leaving groups in biomolecules
- Use acid-base and resonance reasoning to predict steps
- Connect recurring reaction families to biological examples

Navigate this guide

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Rebuilding the Foundational Knowledge That Makes Every Mechanism Transparent

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PART I: THE ORGANIC CHEMISTRY YOU NEED TO HAVE BACK

This section rebuilds the essential organic chemistry concepts from the ground up. Every mechanism in this guide is built from these primitives. If you internalize this section, the mechanisms stop being things you learn and start being things you can derive.

1.1 Electrons Are the Only Thing That Matters

Every reaction in organic chemistry and biochemistry is about electrons — where they are, where they want to go, and what drives them to move. Curved arrows are the notation system for tracking electron movement. The tail of a curved arrow always starts where the electrons currently are (a lone pair, a bond), and the head of the arrow always points to where the electrons are going (an atom that wants electrons, or a position where a new bond will form). A full curved arrow (two-barbed head) represents the movement of two electrons. A half-arrow (fishhook, one-barbed head) represents one electron, but you will not encounter single-electron mechanisms in this guide.

When you draw a mechanism, you are telling a story about electrons. Every arrow must have a chemical reason — electrons move because there is a driving force (an electrophilic atom pulling them, a leaving group departing, a base removing a proton). If you cannot articulate why the electrons move in a given step, the arrow is wrong.

1.2 Nucleophiles and Electrophiles

A nucleophile is an electron-rich species that donates a pair of electrons to form a new bond. Nucleophiles are attracted to positive or electron-poor centers. Common nucleophiles in this guide include: thiolates (R–S^-, the deprotonated form of a thiol), alkoxides (R–O^-), carbanions (a carbon bearing a lone pair or excess electron density), enolates (a specific type of carbanion, discussed below), water, amines (R–NH_2, which have a lone pair on nitrogen), and the C2 carbanion of TPP.

An electrophile is an electron-poor species that accepts a pair of electrons to form a new bond. Electrophiles are attracted to negative or electron-rich centers. Common electrophiles in this guide include: carbonyl carbons (the carbon of a C=O group, which is electron-poor because oxygen pulls electron density away), phosphorus atoms in phosphate groups (surrounded by electron-withdrawing oxygens), protons (H^+, the simplest electrophile), and the sulfur atoms in a disulfide bond (mildly electrophilic because the S–S bond is polarizable).

The fundamental event in most mechanisms is a nucleophile attacking an electrophile. The curved arrow goes from the nucleophile (source of electrons) to the electrophile (destination of electrons). When a nucleophile attacks a carbonyl carbon, the two electrons in the C=O π bond must go somewhere — they get pushed onto the oxygen as a lone pair. This is why carbonyl additions always show two simultaneous arrows: one from the nucleophile to the carbonyl carbon, and one from the C=O bond to the oxygen.

1.3 What a Carbonyl Is and Why It Matters So Much

A carbonyl group is a carbon double-bonded to an oxygen: C=O. Oxygen is much more electronegative than carbon, so it pulls the shared electrons of the double bond toward itself. This creates a permanent dipole: the carbon is partially positive (δ^+, electron-poor, electrophilic) and the oxygen is partially negative (δ^-, electron-rich). This polarity is the reason carbonyl carbons are reactive — they are electrophilic centers that attract nucleophiles.

Different functional groups contain a carbonyl, and their reactivity differs based on what else is bonded to the carbonyl carbon:

An **aldehyde** has the carbonyl carbon bonded to one hydrogen and one carbon chain: R–C(=O)–H. The hydrogen makes it relatively accessible to nucleophilic attack. Aldehydes are moderately reactive electrophiles.

A **ketone** has the carbonyl carbon bonded to two carbon chains: R–C(=O)–R'. Ketones are slightly less reactive than aldehydes because the two R groups provide modest steric hindrance and donate electron density through induction.

A **carboxylic acid** has the carbonyl carbon bonded to a hydroxyl group: R–C(=O)–OH. At physiological pH, this is deprotonated to a **carboxylate**: R–C(=O)–O^-. The negative charge on the carboxylate is stabilized by resonance — the two C–O bonds are equivalent, with the negative charge delocalized

across both oxygens. This resonance stabilization makes carboxylates very poor electrophiles — they are reluctant to react with nucleophiles because the negative charge repels incoming electron-rich species and the resonance makes the carbonyl carbon less electrophilic.

An **ester** has the carbonyl carbon bonded to an –OR group: R–C(=O)–O–R'. The oxygen lone pairs donate into the carbonyl through resonance, reducing the electrophilicity of the carbonyl carbon compared to an aldehyde or ketone. Esters are moderately stable.

A **thioester** has the carbonyl carbon bonded to an –SR group: R–C(=O)–S–R'. Sulfur is larger than oxygen and its lone pairs overlap much less effectively with the carbonyl's π system. This means the resonance stabilization that makes esters relatively unreactive is significantly weakened in thioesters. The result is that thioesters are more reactive than esters — the carbonyl carbon is more electrophilic, and the C–S bond is easier to break. Thioesters are "high-energy" bonds in metabolism precisely because they are less resonance-stabilized, meaning their hydrolysis releases more free energy than ordinary ester hydrolysis. Acetyl-CoA is the most important thioester in this guide.

An **acyl phosphate** has the carbonyl carbon bonded to a phosphate group through an oxygen: R–C(=O)–O–PO_3^{2-}. This is a mixed anhydride between a carboxylic acid and phosphoric acid. Acyl phosphates are very reactive (high-energy) because both the carboxylate and the phosphate products of hydrolysis are stabilized by resonance and hydration. 1,3-BPG contains an acyl phosphate at C1.

An **amide** has the carbonyl carbon bonded to a nitrogen: R–C(=O)–NH–R'. Nitrogen donates its lone pair into the carbonyl very effectively (even more than oxygen), making amides the least reactive common carbonyl derivatives. Peptide bonds are amides. The linkage between biotin and its lysine residue is an amide.

An **imine** (also called a Schiff base) has a carbon double-bonded to a nitrogen instead of an oxygen: R–C(=N–R')–R". Imines are important because the nitrogen lone pair can accept a proton, making the imine nitrogen positively charged (an iminium ion, R–C(=N^+H–R')–R"). A protonated imine is a much better electron sink than a regular carbonyl, which is why enzymes like aldolase form Schiff bases — the iminium ion pulls electron density away from the adjacent carbon more effectively than the original ketone did, stabilizing developing negative charges during C–C bond cleavage.

1.4 What a Carbanion Is

A carbanion is a carbon atom bearing a formal negative charge — it has a lone pair of electrons that is not shared with any other atom. Carbanions are extremely unstable in isolation because carbon is not very electronegative and does not stabilize negative charge well. Naked carbanions essentially do not exist in biological systems; they require some form of stabilization to exist even transiently.

The ways a carbanion can be stabilized are central to understanding when cofactors are required:

Resonance stabilization by an adjacent carbonyl (→ enolate): If the carbanion is on a carbon directly adjacent to a carbonyl group (the α-carbon), the negative charge can be delocalized into the carbonyl through resonance. The electrons on the carbanion carbon flow into the C–C bond between the α-carbon and the carbonyl carbon, pushing the C=O π electrons onto the oxygen. The result is an

enolate: a species with negative charge distributed between the α-carbon and the carbonyl oxygen. Enolates are moderately stable — stable enough to exist as reactive intermediates in enzyme active sites without any cofactor assistance.

Resonance stabilization by a protonated imine (→ enamine): If the carbanion is on a carbon directly adjacent to a protonated imine ($C{=}N^{+}H{-}R$), the negative charge can be delocalized into the C=N bond in the same way it delocalizes into a C=O bond in an enolate. The resulting species is called an enamine. This is the basis of the aldolase Schiff base mechanism — the enzyme replaces the substrate's ketone with an imine (by reacting with an active-site lysine), creating a more effective electron sink for stabilizing the carbanion during C–C bond cleavage.

Stabilization by the thiazolium ring of TPP: If the carbanion is generated at a position where there is no adjacent carbonyl, no adjacent imine, and no other means of resonance stabilization, then the carbanion would be naked and hopelessly unstable. TPP solves this problem by pre-bonding to the substrate at the carbon where the carbanion will form, so that when the C–C bond breaks and the carbanion is generated, the electrons flow directly into the thiazolium ring. The positively charged nitrogen of the thiazolium ring and the adjacent sulfur atom delocalize the negative charge through the ring system. TPP is, in essence, an externally supplied electron sink for carbanions that have no internal means of stabilization.

This is the core decision framework for TPP: Does the carbanion generated by C–C bond cleavage have an adjacent carbonyl (or imine) to delocalize into? If yes, no TPP needed — the enolate (or enamine) is sufficient. If no, TPP is required.

1.5 What an Enolate Is and How to Recognize When One Will Form

An enolate is the resonance-stabilized carbanion formed when a proton is removed from the α-carbon (the carbon adjacent to a carbonyl). The two resonance structures of an enolate are: (1) the negative charge on the α-carbon with the C=O intact, and (2) the negative charge on the oxygen with a C=C double bond between the α-carbon and the carbonyl carbon. Both resonance structures contribute to the actual electronic structure, meaning the negative charge is spread across both the carbon and the oxygen.

To determine whether an enolate can form in a given situation, ask: is there a hydrogen on the carbon adjacent to a carbonyl? If yes, a base can remove that proton to generate an enolate. If the carbon adjacent to the carbonyl has no hydrogens (it is quaternary, or it has no α-carbon at all), an enolate cannot form at that position.

Enolates are nucleophilic. The carbon bearing the partial negative charge can attack electrophiles. This is the basis of aldol reactions (enolate attacks another carbonyl to form a new C–C bond), Claisen condensations (enolate attacks a thioester carbonyl), and many biosynthetic reactions. In pyruvate carboxylase, the methyl group of pyruvate is deprotonated to form an enolate, and this enolate carbon attacks the carboxyl group of carboxybiotin, forming the new C–C bond that creates oxaloacetate.

1.6 The α-Carbon, β-Carbon, and Why Position Matters for TPP

The Greek letter designations for carbons relative to a functional group are critical for the TPP decision. The α-carbon is the carbon directly bonded to the carbonyl carbon. The β-carbon is the next one out. The γ-carbon is the one after that.

In an **α-keto acid** (like pyruvate: $CH_3–CO–COO^-$), the carboxylate group is bonded directly to the carbonyl carbon. When this carboxylate departs as CO_2, the carbanion is generated at the carbonyl carbon itself — there is no adjacent carbonyl on the other side to stabilize it as an enolate. This is the situation that requires TPP.

In a **β-keto acid** (like oxaloacetate: $^-OOC–CH_2–CO–COO^-$, where the C4 carboxylate is β to the C2 ketone), when the carboxylate departs as CO_2, the carbanion is generated at a carbon that is α to the remaining ketone. This carbanion can be stabilized as an enolate by the adjacent carbonyl. No TPP needed.

In a **β-keto acid decarboxylation**, the electrons from the breaking C–C bond flow toward the β-carbon, generating a carbanion there. But because there is a carbonyl at the α-position (one carbon further in), those electrons can continue to delocalize into the C=O bond, forming an enolate. The carbonyl acts as an internal electron sink. In an **α-keto acid decarboxylation**, the carbanion is generated right at the carbonyl carbon, and there is no second carbonyl available to form an enolate. The only electron sink available is the externally provided thiazolium ring of TPP.

To apply this in practice: when you see a decarboxylation, identify the carbon where the carbanion will form after CO_2 leaves. Look at that carbon's neighbors. If there is a carbonyl group adjacent to it (one bond away), the carbanion can form an enolate and no TPP is needed. If there is no adjacent carbonyl, TPP is needed. This is why PEPCK (OAA → PEP + CO_2) does not need TPP but pyruvate dehydrogenase (pyruvate → acetyl unit + CO_2) does.

1.7 Oxidation States of Carbon — How to Track Them

The oxidation state of a carbon atom in an organic molecule is determined by counting bonds to atoms that are more electronegative than carbon (primarily oxygen, nitrogen, sulfur, and halogens) versus bonds to atoms that are less electronegative or equal (primarily hydrogen and other carbon atoms).

The simplest rule: each bond from carbon to a more electronegative atom (O, N, S) raises the oxidation state by one unit. Each bond from carbon to hydrogen lowers it by one unit. Bonds from carbon to carbon do not change the oxidation state.

Working through the functional groups on a single carbon:

A **methane carbon** (CH_4): four bonds to H → most reduced state. Oxidation state = -4 (or just think of it as "fully reduced").

A **methanol carbon** (–CH_2OH): one bond to O, rest to H/C → one oxidation step up from fully reduced. Alcohol oxidation state.

A **thiol carbon** (–CH_2SH): same oxidation state as the alcohol carbon — sulfur and oxygen count the same way.

An **aldehyde carbon** (–CHO): one C=O (counts as two bonds to O), one C–H. Two steps oxidized from the alcohol. Alternatively, compare: the alcohol carbon has one bond to O and two to H; the aldehyde carbon has two bonds to O and one to H. The aldehyde is more oxidized.

A **carboxylic acid carbon** (–COOH or $–COO^-$): three bonds to oxygen. Three steps oxidized from the alcohol. This is also the oxidation state of an ester (–COOR), a thioester (–COSR), and an acyl phosphate ($–CO–OPO_3^{2-}$). All of these functional groups have the carbonyl carbon making three bonds to heteroatoms.

A **CO_2 carbon**: four bonds to oxygen. Fully oxidized.

How to use this for identifying redox reactions: Compare the oxidation state of each carbon in the substrate to the corresponding carbon in the product. If any carbon has increased its number of bonds to oxygen (or decreased bonds to hydrogen) without a compensating change, the substrate has been oxidized. If the reverse, it has been reduced. If no carbon changes oxidation state, the reaction is not redox.

Specific examples from this guide:

Aldehyde → acyl phosphate: The carbonyl carbon goes from two bonds to O and one to H (aldehyde) to three bonds to O (acyl phosphate). This is an oxidation. NAD^+ is needed.

Ketone → alcohol: The carbonyl carbon goes from two bonds to O (ketone) to one bond to O and one to H (alcohol). This is a reduction. NADH is needed.

Carboxylate → acyl phosphate: The carbonyl carbon stays at three bonds to O in both the carboxylate and the acyl phosphate. The oxygen partner changed ($–O^-$ became $–O–PO_3^{2-}$), but the oxidation state of carbon did not change. Not redox. No NAD^+ needed. ATP is needed for the phosphoryl transfer.

Disulfide → two thiols: No carbon changes. The sulfur atoms change oxidation state (sulfur in a disulfide is more oxidized than sulfur in a thiol). This is a reduction of sulfur. FAD is involved.

1.8 Leaving Groups

A leaving group is the atom or group of atoms that departs with a pair of electrons when a bond to it is broken. Good leaving groups are stable after they depart — they can accommodate the negative charge without being excessively basic.

Common leaving groups in this guide:

Thiolates (R–S^-): Sulfur is large and polarizable, which stabilizes the negative charge. Thiolates are excellent leaving groups. This is why thioesters are reactive — the thiolate departing from a thioester is a stable species, making the bond easy to break. When CoA–SH attacks acetyl-lipoamide, the lipoamide thiolate departs as the leaving group.

Phosphate and pyrophosphate: Pi (HPO_4^{2-}) and PPi ($P_2O_7^{4-}$) are outstanding leaving groups because their negative charges are stabilized by extensive resonance delocalization across multiple oxygen atoms.

CO_2: Carbon dioxide is an exceptional leaving group in decarboxylations. Once CO_2 departs, it is a stable, low-energy molecule that does not readily re-attach. The loss of CO_2 is effectively irreversible, which makes decarboxylations thermodynamically favorable.

Water: In dehydration reactions (like enolase removing water from 2-PG to form PEP), water departs as a neutral molecule. A general acid typically protonates the departing hydroxyl to help it leave as water rather than as hydroxide (which would be a poor leaving group).

Amines: In Schiff base hydrolysis, the amine (lysine ε-amino group) departs after water attacks the iminium carbon. The amine is protonated upon departure, making it a neutral or cationic leaving group.

1.9 General Acid and General Base Catalysis

Enzymes accelerate reactions by providing catalytic residues that donate or accept protons at precisely the right moment. A general base (:B) is a residue that accepts a proton from the substrate, making the substrate more nucleophilic or facilitating a bond change. A general acid (H–A or $H–B^+$) is a residue that donates a proton to the substrate, stabilizing a developing negative charge or making a group a better leaving group.

In every mechanism you draw, you must explicitly show when protons are transferred and identify the general acid or base responsible. The mechanism-writing convention is that you can use the generic labels ":B" or "B:" for a general base and "$H–B^+$" or "H–A" for a general acid without specifying the actual amino acid residue. A complete mechanism includes every protonation and deprotonation event.

When to include a general base:

- When a thiol (R–SH) needs to be deprotonated to a thiolate (R–S^-) before it can act as a nucleophile
- When the C2–H of TPP needs to be removed to form the ylide
- When an α-proton adjacent to a carbonyl is removed to form an enolate
- When a hydroxyl is being deprotonated prior to nucleophilic attack

When to include a general acid:

- When an oxygen develops a negative charge during nucleophilic attack on a carbonyl (the alkoxide must be protonated to form a stable hydroxyl)

- When a leaving group needs to be protonated to depart (e.g., water leaving in a dehydration, amine leaving in Schiff base hydrolysis)
- When the product of a reduction needs its new oxygen protonated (as in LDH: the alkoxide formed by hydride delivery to pyruvate must be protonated to form lactate's hydroxyl)

1.10 The Tetrahedral Intermediate

When a nucleophile attacks a carbonyl carbon, the trigonal planar geometry of the carbonyl (three groups arranged flat, 120° apart) changes to tetrahedral geometry (four groups arranged in 3D, 109.5° apart) because a fourth group (the nucleophile) has bonded to the carbon. The carbon can no longer support a double bond to oxygen — the π bond electrons are pushed onto the oxygen as a lone pair. This tetrahedral species is called the tetrahedral intermediate.

Tetrahedral intermediates are transient — they collapse back to a trigonal planar species by expelling a leaving group. The leaving group departs with the electron pair from its bond to the central carbon, and the C=O double bond re-forms. Which group departs depends on leaving group ability. In a thioester, the thiolate can depart; in an ester, the alkoxide can depart; in an acyl phosphate, the phosphate can depart.

This pattern — nucleophilic attack → tetrahedral intermediate → leaving group departure — is called the **nucleophilic acyl substitution** mechanism. It is the mechanism for all acyl transfer reactions: transesterifications (acetyl-lipoamide → acetyl-CoA), the phosphorolysis step of GAPDH (thioester → acyl phosphate), and hydrolysis of esters and amides.

You will see this pattern repeatedly: nucleophile attacks carbonyl → C=O electrons go to oxygen → tetrahedral intermediate forms → a different group departs as the leaving group → C=O reforms. It is one mechanism applied to many different substrates.

PART II: MECHANISM CONSTRUCTION — EVERY TYPE, WITH FOUNDATIONS VISIBLE

For each mechanism type below, I will identify the reaction, explain how to recognize it, state what is always the same regardless of the specific substrate, explain the driving force, and then walk through the construction step by step with the foundational organic chemistry visible at each step.

Mechanism Type 1: TPP-Catalyzed Decarboxylation

How to Recognize

You have a substrate that will lose CO_2. Examine the carbon where the carbanion will form after CO_2 departs. Does that carbon have an adjacent carbonyl to form an enolate? If no → TPP is required. The substrate is almost always an α-keto acid (the carboxylate is directly bonded to the keto carbon: R–CO–

COO^-), though it can also be any situation where C–C bond cleavage generates a carbanion without an adjacent electron sink.

What Is Always the Same

The catalytic cycle always starts with ylide formation (deprotonation of C2 on the thiazolium ring), always involves nucleophilic attack by the ylide on the substrate's carbonyl carbon, always involves decarboxylation (or C–C cleavage) with electrons flowing into the thiazolium ring, and always ends with the product departing and TPP being regenerated. These four events are invariant — the only thing that changes between different TPP enzymes is what happens to the carbanion intermediate after decarboxylation (is it protonated and released? transferred to lipoamide? used to attack another substrate?).

Step-by-Step Construction

Step 1 — Ylide formation.

The thiazolium ring of TPP contains a nitrogen (positively charged), a sulfur, and a C2 carbon that bears a proton in its resting state. This C2–H proton is unusually acidic because the resulting carbanion at C2 is stabilized by two features: the adjacent positively charged nitrogen (which electrostatically stabilizes the negative charge) and the adjacent sulfur (which stabilizes negative charge through polarizability and d-orbital overlap). A general base in the active site (draw as ":B") removes this proton.

Arrow: from the C2–H bond to the base. This generates the TPP ylide — a nucleophilic carbanion at C2. Write "ylide" next to this species.

Step 2 — Nucleophilic attack on the substrate.

The TPP ylide (nucleophile) attacks the electrophilic carbonyl carbon of the substrate. For pyruvate, this is the C2 (the keto carbon between the methyl and the carboxylate). The carbonyl carbon is electrophilic because the C=O bond is polarized, placing partial positive charge on carbon.

Arrow 1: from the C2 lone pair of the TPP ylide to the carbonyl carbon of the substrate. This forms a new C–C bond between TPP and the substrate.

Arrow 2 (simultaneous): from the C=O π bond electrons of the substrate to the oxygen, converting C=O to $C–O^-$. This oxygen will be protonated by a general acid in the next step, or it may remain as an alkoxide temporarily.

Arrow 3 (if applicable): a general acid ($H–B^+$) protonates the developing alkoxide. Arrow from the H–B bond to the oxygen.

The product is a tetrahedral addition compound with four groups on the former keto carbon: the TPP (via the new C–C bond), the methyl group, the carboxylate, and the hydroxyl (or alkoxide).

Step 3 — Decarboxylation.

This is the key step. The C–C bond between the substrate's former keto carbon and the carboxylate carbon breaks. CO_2 departs as a leaving group.

Arrow 1: from the C–C bond (the one connecting to the carboxylate) toward the carboxylate group. This pushes the electrons into the CO_2, which departs. Two drawing conventions are common: show a curved arrow from the C–C bond into a forming C=O bond, or depict CO_2 departure directly.

Arrow 2 (simultaneous or immediately following): The pair of electrons that previously formed the C–C bond to the carboxylate now needs a home. They flow in the opposite direction — toward the TPP ring. Specifically, they delocalize through the bond connecting the substrate carbon to the TPP C2, and into the thiazolium ring. Draw the arrow from the position of the breaking C–C bond toward the N^+ of the thiazolium ring (showing electron delocalization into the ring). The positive nitrogen and the ring system absorb this electron density, stabilizing what would otherwise be a naked carbanion.

The product is the resonance-stabilized carbanion on TPP. In the pyruvate case, this species is called **hydroxyethyl-TPP**: the C2 of TPP is bonded to a carbon that bears a hydroxyl and a methyl group, with negative charge delocalized into the thiazolium ring.

Step 4 — Divergence (what happens to the carbanion).

Variant A — Pyruvate decarboxylase (yeast): The carbanion is protonated by a general acid ($H–B^+$). Arrow from H–B to the carbanion carbon. Then the C–C bond between TPP and the product breaks: arrow from this bond toward the product carbon, releasing acetaldehyde. The TPP ylide is regenerated (or it is protonated and must be re-deprotonated in the next cycle).

Variant B — Pyruvate dehydrogenase E1: The carbanion is not protonated. Instead, oxidized lipoamide approaches. Lipoamide's disulfide bond (S–S) is mildly electrophilic — the sulfur atoms are targets for nucleophilic attack. The carbanion on hydroxyethyl-TPP attacks one of the lipoamide sulfur atoms. Arrow from the carbanion electron density to one sulfur. The S–S bond of lipoamide breaks: arrow from the S–S bond to the other sulfur (generating a thiolate at that sulfur). Simultaneously, the two-carbon fragment is transferred from TPP to lipoamide.

During this transfer, the two-carbon fragment is oxidized: it goes from the hydroxyl/alcohol oxidation state (hydroxyethyl-TPP) to the thioester oxidation state (acetyl-lipoamide). The lipoamide sulfur that accepted the fragment is now bonded to the acetyl group through a thioester linkage, and the other sulfur is now a free thiol. TPP's C–C bond to the fragment breaks (arrow from bond to TPP, regenerating the ylide). A general acid protonates the thiolate.

Variant C — Acetolactate synthase: The carbanion from the first pyruvate decarboxylation acts as a nucleophile attacking the carbonyl carbon of a second pyruvate molecule. Arrow from the carbanion to the keto carbon of the second pyruvate. Arrow from the C=O of the second pyruvate to the oxygen. A new C–C bond forms. The product (a four-carbon species, acetolactate with CO_2 having been lost from the first pyruvate) is released from TPP.

Common Error Points

Forgetting to draw the ylide formation step. Drawing CO_2 loss without showing where the electrons go (they must go into the thiazolium ring — this is the entire reason TPP exists). Forgetting to regenerate TPP at the end. Not showing general acid/base catalysis for protonation and deprotonation events.

Mechanism Type 2: GAPDH-Type Oxidative Phosphorylation (Aldehyde → Acyl Phosphate via NAD^+)

How to Recognize

The substrate has an aldehyde (R–CHO, a carbon making a double bond to O and a single bond to H). The product is an acyl phosphate (R–CO–O–PO_3^{2-}, the same carbon now making three bonds to oxygen atoms). No ATP is consumed. NAD^+ and Pi are substrates. The driving force is the oxidation of the aldehyde — the change from two bonds to O to three bonds to O releases enough energy to form the high-energy acyl phosphate bond.

What Is Always the Same

There is always a cysteine thiol in the active site that forms a covalent intermediate. There is always a two-stage process: first oxidation (NAD^+ accepts a hydride, forming a thioester), then phosphorolysis (Pi displaces the cysteine from the thioester, forming the acyl phosphate). The thioester intermediate is the crucial energy-storing species that makes the whole reaction possible.

Step-by-Step Construction

Step 1 — Cysteine thiol activation and nucleophilic attack.

The active-site cysteine bears a thiol group (–SH). A general base (":B") deprotonates this thiol to generate a thiolate (–S^-). The thiolate is a strong nucleophile (sulfur is large, polarizable, and carries a full negative charge).

Arrow 1: from the S–H bond to the base.

The thiolate attacks the aldehyde carbonyl carbon. The aldehyde carbon is electrophilic (partial positive charge from the C=O dipole). The nucleophilic thiolate sulfur forms a new C–S bond with this carbon.

Arrow 2: from the sulfur lone pair to the aldehyde carbonyl carbon. Arrow 3 (simultaneous): from the C=O π bond to the oxygen (generating an alkoxide or protonated by a general acid).

The product of this step is a **hemithioacetal** — a tetrahedral intermediate where the central carbon is bonded to the enzyme sulfur, an –OH (or –O^-), a hydrogen, and the rest of the substrate. This is not yet at the thioester stage.

Step 2 — Oxidation by NAD^+ (hemithioacetal → thioester).

NAD^+ now oxidizes the hemithioacetal to a thioester. This is the key redox event. The C–H bond on the central carbon (the hydrogen that was part of the original aldehyde) is the source of the hydride. The hydride (H^- = the hydrogen atom plus both electrons from the C–H bond) transfers to the C4 position of the nicotinamide ring of NAD^+.

Arrow 1: from the C–H bond of the hemithioacetal to the C4 of NAD^+'s nicotinamide ring. This delivers two electrons and a proton to NAD^+, reducing it to NADH.

Arrow 2 (simultaneous): the electrons that were in the C–OH bond (the hydroxyl on the hemithioacetal) re-form the C=O double bond. Arrow from the oxygen lone pair back toward the carbon, re-establishing the carbonyl. This converts the hemithioacetal into a thioester (Enz–S–C(=O)–R).

NADH departs from the active site. A fresh NAD^+ binds (this exchange of NADH for NAD^+ must occur before the next step).

At this point, the carbon that started as an aldehyde is now a thioester — it has been oxidized from two bonds to O to the equivalent of three bonds to O (because a thioester has the same oxidation state as a carboxylic acid). The energy of this oxidation is stored in the thioester bond, which is a high-energy bond due to the poor resonance stabilization by sulfur.

Step 3 — Phosphorolysis (thioester → acyl phosphate).

Inorganic phosphate (Pi, drawn as HPO_4^{2-} or $H_2PO_4^-$) attacks the thioester carbonyl carbon. Pi is the nucleophile; the thioester carbonyl carbon is the electrophile.

Arrow 1: from an oxygen lone pair of Pi to the carbonyl carbon of the thioester. Arrow 2 (simultaneous): from the C=O π bond to the oxygen (forming a tetrahedral intermediate).

The tetrahedral intermediate then collapses: the C–S bond to the cysteine breaks, because the thiolate is a good leaving group.

Arrow 3: from the C–S bond to the sulfur (cysteine thiolate departs). Arrow 4 (simultaneous): the C=O double bond re-forms.

The product is the acyl phosphate (1,3-BPG in the case of glycolysis) and the free enzyme cysteine (which may be reprotonated by a general acid).

Why This Mechanism Does Not Require ATP

The energy for forming the acyl phosphate bond comes from the oxidation of the aldehyde, captured via the thioester intermediate. ATP is not needed because the substrate itself provides the energy through its change in oxidation state. This is the fundamental distinction from Decision 5 (carboxylate → acyl phosphate), where the substrate is already fully oxidized and has no energy to give.

Mechanism Type 3: Hydride Transfer (LDH, Reduction of a Ketone to an Alcohol)

How to Recognize

One substrate has a ketone (C=O). The product has an alcohol (C–OH) at that same carbon. NADH is consumed (oxidized to NAD^+). This is a simple reduction — the ketone accepts a hydride from NADH and a proton from a general acid.

What Is Always the Same

The hydride always comes from the C4 position of NADH's nicotinamide ring. The hydride always attacks the carbonyl carbon. A general acid always protonates the developing alkoxide on the oxygen. The stereochemistry is controlled by the enzyme (LDH produces L-lactate specifically).

Step-by-Step Construction

Step 1 — Hydride delivery.

NADH has a C4–H bond on its nicotinamide ring. This hydrogen, along with both electrons from the C–H bond (making it H^-, a hydride), transfers to the electrophilic C2 carbonyl carbon of pyruvate.

Arrow 1: from the C4–H bond of NADH to the C2 carbon of pyruvate. This forms a new C–H bond on the pyruvate carbon.

Arrow 2 (simultaneous): from the C=O π bond of pyruvate to the oxygen. The oxygen picks up the electron pair as a lone pair, becoming an alkoxide ($C–O^-$).

NADH has now been oxidized to NAD^+ (it lost a hydride, which means it lost two electrons).

Step 2 — Protonation of the alkoxide.

The alkoxide is unstable — it needs to be protonated to become the stable hydroxyl of lactate. A general acid ($H–B^+$) donates a proton to the oxygen.

Arrow: from the H–B bond to the oxygen lone pair of the alkoxide. The oxygen is now –OH. The product is L-lactate.

For the Reverse Direction (Lactate → Pyruvate, as in Gluconeogenesis)

Everything runs backward. A general base (:B) removes the proton from the C2 hydroxyl of lactate (arrow from O–H bond to the base, generating $C–O^-$). The electrons from the C–O bond reform the C=O double bond (arrow from oxygen lone pair to the C–O bond). Simultaneously, the C–H bond on C2 breaks and the hydride transfers to NAD^+ at C4 (arrow from the C–H bond to C4 of NAD^+). The product is pyruvate and NADH.

Mechanism Type 4: Disulfide Exchange (Thiol-Disulfide Interchange)

How to Recognize

You see two thiol groups (–SH, –SH) being converted to a disulfide (–S–S–), or a disulfide being converted to two thiols. Sulfur atoms are changing partners. This is not hydride chemistry — it is nucleophilic displacement chemistry at sulfur.

The Foundational Chemistry

A disulfide bond (S–S) is a covalent bond between two sulfur atoms. It is moderately electrophilic — a nucleophilic thiolate ($R–S^-$) can attack one sulfur of a disulfide, displacing the other sulfur as a thiolate leaving group. This is a direct analogy to an S_n2 reaction: the nucleophile (thiolate) attacks the electrophilic center (one sulfur of the disulfide), and the other sulfur departs with the bonding electrons. The result is a new disulfide bond between the attacking sulfur and the sulfur it attacked, and a free thiolate from the sulfur that was displaced.

This is called thiol-disulfide exchange, and it is the mechanism by which lipoamide transfers its electrons to the E3 cysteine residues in the PDH complex.

What Is Always the Same

A thiolate (deprotonated thiol, S^-) is always the nucleophile. A disulfide sulfur is always the electrophile. The other sulfur of the disulfide is always the leaving group. Each exchange step involves one nucleophilic attack and one leaving group departure.

Step-by-Step Construction (Dihydrolipoamide Reducing E3 Cystine)

Step 1 — First exchange.

A general base deprotonates one thiol of dihydrolipoamide (arrow from S–H bond to base), generating a thiolate (S^-).

This thiolate attacks one sulfur of the E3 active-site disulfide (cystine). Arrow from the lipoamide thiolate lone pair to one sulfur of the cystine. The S–S bond of the cystine breaks: arrow from the S–S bond to the other cysteine sulfur. That cysteine sulfur becomes a thiolate (S^-), which is protonated by a general acid (arrow from H–A to the cysteine sulfur).

Result: a mixed disulfide between one lipoamide sulfur and one cysteine sulfur. One cysteine is now a free thiol. One lipoamide sulfur is in the disulfide; the other is still a free thiol.

Step 2 — Second exchange.

A general base deprotonates the second lipoamide thiol (arrow from S–H to base), generating a second thiolate.

This thiolate attacks the lipoamide sulfur that is involved in the mixed disulfide. Arrow from the second lipoamide thiolate to the first lipoamide sulfur (the one in the mixed disulfide). The bond between the first lipoamide sulfur and the cysteine sulfur breaks: arrow from that S–S bond to the cysteine sulfur (generating a cysteine thiolate, protonated by a general acid).

Result: lipoamide has reformed its own internal disulfide (now re-oxidized), and both E3 cysteines are in the reduced (thiol) form. The lipoamide is now ready for the next catalytic cycle of the PDH complex, while the E3 cysteines must be re-oxidized by FAD.

Why NAD^+ Cannot Do This Directly

This is worth understanding deeply because it explains why FAD exists as an intermediary. NAD^+ accepts hydride ions (H^-) — that is, a hydrogen atom with both bonding electrons from a C–H bond. The hydride is delivered to the C4 position of the nicotinamide ring. This is specifically two-electron, hydrogen-atom-transfer chemistry at carbon.

Thiol-disulfide exchange does not produce or consume hydrides. The electrons move between sulfur atoms through nucleophilic displacement, not through hydride transfer. There is no step in the disulfide exchange mechanism where a hydrogen with both electrons from a bond is transferred. The electrons are shuffled between sulfur atoms, not packaged as a hydride. NAD^+ has no mechanism for accepting electrons from sulfur-based redox chemistry.

FAD bridges this gap because its isoalloxazine ring can accept electrons through covalent chemistry at its C4a position (a sulfur from the reduced cysteine forms a transient covalent bond to C4a of FAD), and the resulting reduced form ($FADH^-$) can then donate a hydride to NAD^+. FAD is chemically versatile in a way that NAD^+ is not — it can interface with both sulfur-based and hydride-based electron transfer.

Mechanism Type 5: FAD Regeneration ($FADH^-$ → FAD + NADH)

How to Recognize

You are told that $FADH^-$ (the anionic, resonance-stabilized reduced form of FAD) must be re-oxidized. NAD^+ is the terminal electron acceptor. This occurs at the E3 stage of the PDH complex.

The Foundational Chemistry

$FADH^-$ is a hydride donor. The key hydrogen is at N5 of the reduced isoalloxazine ring. When this hydride (the N5 hydrogen plus two electrons) transfers to NAD^+, the isoalloxazine ring is re-oxidized to its fully conjugated, aromatic FAD form. NAD^+ accepts the hydride at C4, becoming NADH.

Step-by-Step Construction

Step 1 — Hydride transfer.

Arrow: from the N5–H bond of $FADH^-$ to the C4 of the nicotinamide ring of NAD^+. The two electrons and the hydrogen atom transfer together as a hydride.

As the hydride departs from $FADH^-$, the electrons in the isoalloxazine ring system rearrange to restore the fully oxidized, conjugated FAD structure. NAD^+ becomes NADH. FAD is regenerated.

This is mechanistically simple — it is essentially the same hydride transfer that occurs in LDH, except the hydride source is a flavin nitrogen rather than a substrate carbon. The important conceptual point is not the mechanism itself (which is just one arrow) but why this step exists in the larger context (because the electrons from dihydrolipoamide could not reach NAD^+ without FAD as an intermediary).

Mechanism Type 6: Schiff Base C–C Bond Cleavage (Aldolase Type)

How to Recognize

A C–C bond is broken in a substrate, and one fragment contains a carbonyl that has been (or could be) converted to a Schiff base with an active-site lysine. The carbanion generated by the cleavage is stabilized as an enamine (the Schiff base equivalent of an enolate). No external cofactor like TPP is needed because the Schiff base provides the electron sink.

The Foundational Chemistry — Why a Schiff Base Instead of Just the Ketone

The substrate (F-1,6-BP in aldolase) has a ketone at C2. In principle, the carbonyl oxygen of this ketone could stabilize the carbanion formed at C3 during retro-aldol cleavage — as a simple enolate. However, enzymes like aldolase convert the ketone to a Schiff base (imine with lysine) because the protonated imine nitrogen ($–C=N^+H–$) is a better electron sink than the carbonyl oxygen (–C=O). Nitrogen is less electronegative than oxygen, so when it is protonated and positively charged, it pulls electron density more effectively. The enhanced electron-sink capability of the protonated imine lowers the activation energy for the retro-aldol cleavage, making the enzyme faster than it would be with the unmodified ketone.

What Is Always the Same

Schiff base formation (substrate ketone + lysine amine → imine + H_2O). Then C–C bond cleavage with electrons flowing into the imine as an enamine. Then Schiff base hydrolysis (water attacks the iminium to release the product and regenerate the free lysine). General acids and bases participate at every protonation/deprotonation step.

Step-by-Step Construction

Step 1 — Schiff base formation.

The ε-amino group of the active-site lysine ($–NH_2$) is the nucleophile. The C2 ketone of F-1,6-BP is the electrophile.

Arrow 1: from the nitrogen lone pair of lysine to the C2 carbonyl carbon of the substrate. Arrow 2 (simultaneous): from the C=O π bond to the oxygen.

This forms a tetrahedral carbinolamine intermediate (carbon bonded to –OH, –NH–Lys, and the two substrate chains). Water is then lost: a general acid protonates the hydroxyl to make it a better leaving group, and water departs. The C–N bond becomes a C=N double bond (the imine or Schiff base). Under enzyme conditions, the imine nitrogen is protonated, forming the iminium ion ($C=N^+H–Lys$).

Step 2 — Retro-aldol C–C bond cleavage.

The C3–C4 bond of the substrate breaks. The C4–C6 fragment (GAP, an aldehyde) departs as a product.

Arrow 1: from the C3–C4 bond toward C3, generating electron density at C3. But this electron density does not remain as a naked carbanion — it immediately delocalizes.

Arrow 2 (simultaneous): the electrons flow from C3 into the C2–C3 bond, and from there into the $C2=N^+$ bond, pushing the positive charge off the nitrogen. This forms an enamine (C2=C3 double bond, with the nitrogen now neutral and bearing a lone pair). The enamine is the stabilized carbanion equivalent.

A general base may abstract a proton from C3 to facilitate this step, or the electron flow may be concerted with bond cleavage.

Step 3 — Protonation of the enamine.

The enamine's C3 carbon is protonated by a general acid ($H–B^+$). Arrow from H–B to C3. This converts the enamine back to the iminium form.

Step 4 — Schiff base hydrolysis (product release).

Water attacks the iminium carbon (C2).

Arrow 1: from a water oxygen lone pair to C2. Arrow 2: from the $C=N^+$ bond to nitrogen. (This breaks the double bond, forming a tetrahedral carbinolamine.)

The C–N bond then breaks: the lysine amine departs as the leaving group.

Arrow 3: from the C–N bond to nitrogen (lysine amine departs, may be protonated). Arrow 4: the C–O bond reforms as C=O. The product (DHAP) is released with its C2 ketone restored. The free lysine is regenerated.

Mechanism Type 7: PEPCK (β-Keto Acid Decarboxylation + Phosphoryl Transfer)

How to Recognize

Oxaloacetate (a four-carbon dicarboxylic acid with a ketone at C2) loses CO_2 and gains a phosphate from GTP to form PEP. No TPP. The carbanion from decarboxylation is stabilized as an enolate by the adjacent C2 carbonyl.

The Foundational Chemistry — Why No TPP

When the C3–C4 bond breaks and the C4 carboxylate departs as CO_2, the carbanion at C3 is α to the C2 carbonyl. The electrons flow from C3 into the C2=O system, forming an enolate with negative charge on the C2 oxygen. This enolate is perfectly stable without TPP. The key check: after CO_2 leaves, is there an adjacent carbonyl for the carbanion to delocalize into? For OAA, yes (the C2 ketone). For pyruvate, no. That is the entire difference.

Step-by-Step Construction

Step 1 — Decarboxylation.

The C3–C4 bond breaks. CO_2 departs.

Arrow 1: from the C3–C4 bond toward C3. The electrons stay with C3, generating a carbanion. Arrow 2 (simultaneous): from the C3 carbanion into the C2–C3 bond, and from the C2=O π bond to the oxygen. This generates the enolate (C2–C3 double bond, negative charge on the C2 oxygen).

For the departing CO_2: arrow from one of the C=O bonds of the carboxylate showing its departure as CO_2.

Step 2 — Phosphoryl transfer from GTP.

The enolate oxygen (C2 oxygen, bearing the negative charge) is a nucleophile. The γ-phosphorus of GTP is an electrophile (surrounded by electron-withdrawing oxygens).

Arrow 1: from the enolate oxygen lone pair to the γ-phosphorus of GTP. Arrow 2 (simultaneous): from the γ–β bridging P–O bond of GTP to the bridging oxygen (GDP departs as the leaving group).

The product is PEP (the enol phosphate, with phosphate esterified at the C2 oxygen) and GDP.

Mechanism Type 8: Pyruvate Carboxylase (Biotin Carboxylation)

How to Recognize

A carboxylate is being added to a substrate using HCO_3^- as the carbon source. ATP is consumed. Biotin is the CO_2 carrier. The substrate (pyruvate) gains one carbon.

Step-by-Step Construction

Step 1 — Bicarbonate activation.

ATP's γ-phosphoryl group is transferred to an oxygen of bicarbonate.

Arrow 1: from a bicarbonate oxygen lone pair to the γ-phosphorus of ATP. Arrow 2: from the γ–β P–O bond to the bridging oxygen (ADP departs).

Product: carboxyphosphate ($HO–CO–O–PO_3^{2-}$), an unstable mixed anhydride, plus ADP.

Step 2 — Biotin carboxylation.

The N1 nitrogen of the biotin ureido ring (which has a lone pair) attacks the carbonyl carbon of carboxyphosphate.

Arrow 1: from the N1 lone pair to the carbonyl carbon of carboxyphosphate. Arrow 2: from the C–O(PO_3) bond to the phosphate oxygen (Pi departs as the leaving group).

Product: N1-carboxybiotin (CO_2 is now covalently attached to biotin) and Pi.

Step 3 — Carboxyl transfer to pyruvate.

A general base deprotonates the C3 (methyl) of pyruvate. The C3 carbanion is stabilized as an enolate by the C2 carbonyl (arrow from C3 to the C2=O system, generating the enolate with negative charge on oxygen).

The enolate carbon (C3) acts as a nucleophile and attacks the carbonyl carbon of the carboxyl group on carboxybiotin.

Arrow 1: from the C3 carbanion/enolate to the carboxyl carbon of carboxybiotin. Arrow 2: from the C–N bond (between the carboxyl and biotin nitrogen) to nitrogen (biotin departs as the leaving group, regenerated).

Product: oxaloacetate (pyruvate with a new $–COO^-$ at C3) and free biotin.

Mechanism Type 9: Nucleophilic Acyl Substitution (Transesterification)

How to Recognize

An acyl group (R–C(=O)–) transfers from one partner to another. One leaving group departs, one nucleophile attacks the same carbonyl carbon. The carbonyl carbon's oxidation state does not change. Examples: acetyl-lipoamide → acetyl-CoA (thioester exchange), the phosphorolysis step of GAPDH (thioester → acyl phosphate).

What Is Always the Same

Nucleophile attacks the carbonyl carbon → tetrahedral intermediate forms → leaving group departs → carbonyl reforms. The only variation is the identity of the nucleophile and leaving group.

Step-by-Step Construction (Acetyl-Lipoamide → Acetyl-CoA)

Step 1 — CoA thiol activation.

General base deprotonates CoA–SH. Arrow from S–H bond to base. CoA–S^- (thiolate) is the nucleophile.

Step 2 — Nucleophilic attack.

Arrow 1: from CoA thiolate lone pair to the carbonyl carbon of acetyl-lipoamide (a thioester). Arrow 2: from the C=O π bond to the oxygen (tetrahedral intermediate forms).

Step 3 — Leaving group departure.

The lipoamide thiolate is the leaving group. Arrow from the C–S bond (to lipoamide sulfur) to the sulfur. The C=O double bond re-forms (arrow from oxygen lone pair back to the C–O bond). Product: acetyl-CoA and dihydrolipoamide (free thiol at the sulfur that lost the acetyl group).

Mechanism Type 10: Phosphoglycerate Mutase (Phospho-Histidine Ping-Pong)

How to Recognize

A phosphate group moves from one position on a substrate to another position on the same substrate. In glycolysis, the phosphate moves from C3 of 3-PG to C2, producing 2-PG. A phosphorylated histidine in the active site and catalytic 2,3-BPG are involved.

Step-by-Step Construction

Step 1 — Phosphoryl transfer from His to substrate.

The phospho-histidine has a phosphate group bonded to one of the nitrogens of the imidazole ring. The C2 hydroxyl of 3-PG is the nucleophile.

Arrow 1: from the C2 oxygen lone pair of 3-PG to the phosphorus of phospho-His. Arrow 2: from the P–N bond (phosphorus to histidine nitrogen) to the nitrogen (histidine departs).

Product: 2,3-BPG (phosphate at both C2 and C3) and free (unphosphorylated) histidine.

Step 2 — Phosphoryl transfer from substrate back to His.

The unphosphorylated histidine nitrogen is now the nucleophile. The C3 phosphate of 2,3-BPG is the target.

Arrow 1: from the histidine nitrogen lone pair to the C3 phosphorus. Arrow 2: from the P–O bond (phosphorus to C3 oxygen) to the oxygen (C3 oxygen departs, now unphosphorylated).

Product: 2-PG (phosphate only at C2) and regenerated phospho-histidine.

Mg^{2+} is required throughout to coordinate the phosphate groups and reduce charge repulsion during the phosphoryl transfers.

Mechanism Type 11: Dehydration (Enolase: 2-PG → PEP)

How to Recognize

Water is removed from a substrate. A C–H bond and a C–OH bond are broken on adjacent carbons, forming a C=C double bond and releasing H_2O. No change in carbon oxidation state — this is not redox.

Step-by-Step Construction

Step 1 — Proton abstraction.

A general base removes the proton from C2 of 2-PG (the carbon adjacent to the carboxylate). Arrow from the C2–H bond to the base.

The electrons from the C2–H bond delocalize into the carboxylate system (forming a stabilized carbanion at C2, with electron density delocalized through the C1 carboxylate).

Step 2 — Hydroxide departure.

The C3–OH bond breaks. A general acid (H–A) protonates the departing hydroxyl, converting it to water (which is a much better leaving group than hydroxide).

Arrow 1: from H–A to the oxygen of the C3 hydroxyl. Arrow 2: from the C3–O bond to the C2–C3 bond (forming the C2=C3 double bond of the enol).

Product: PEP (with the phosphate still at C2, the enol double bond between C2 and C3) and water.

PART III: UNIVERSAL RULES FOR ALL MECHANISM DRAWINGS

Rule 1: Every arrow must start at electrons and end at an electron-deficient target. Tail = electron source (lone pair, bond). Head = electron destination (electrophilic atom, or destination for lone pair).

Rule 2: Show every proton transfer explicitly. Use ":B" for a general base accepting a proton. Use "H–B^+" or "H–A" for a general acid donating a proton. Draw the curved arrow for each transfer. Missing protonation and deprotonation events is a common source of incomplete mechanisms.

Rule 3: When a nucleophile attacks a carbonyl, always show two arrows. One from the nucleophile to the carbonyl carbon (new bond forming), and one from the C=O π bond to the oxygen (electrons displaced onto oxygen). These are simultaneous.

Rule 4: When a leaving group departs from a tetrahedral intermediate, always show two arrows. One from the C–leaving group bond to the leaving group (bond breaking), and one from the oxygen lone pair back to the C–O bond (C=O reforming). These are simultaneous.

Rule 5: Regenerate the catalyst at the end. For TPP mechanisms, the ylide must be regenerated. For Schiff base mechanisms, the free lysine must be regenerated. For FAD mechanisms, FAD must be regenerated. For phospho-His mechanisms, the phospho-His must be regenerated.

Rule 6: Label key intermediates. Write "ylide," "Schiff base," "enamine," "enolate," "thioester intermediate," "tetrahedral intermediate," "hemithioacetal," "carbanion," "$FADH^-$," etc. This makes the electron flow unambiguous and easier to verify.

Rule 7: Verify oxidation states. After drawing, check: did any carbon change oxidation state? If you claimed a redox reaction, verify that a cofactor (NAD^+, NADH, FAD) accepted or donated the corresponding electrons. If no carbon changed oxidation state, no redox cofactor should be present.

Rule 8: Check atom balance. Every atom in the substrates must appear somewhere in the products. Every electron that moves must end up in a defined location. If CO_2 is lost, those atoms (one C, two O) must be accounted for. If water is consumed or produced, those atoms (two H, one O) must appear or disappear appropriately.

End of Organic Chemistry Foundations and Mechanism Construction Guide